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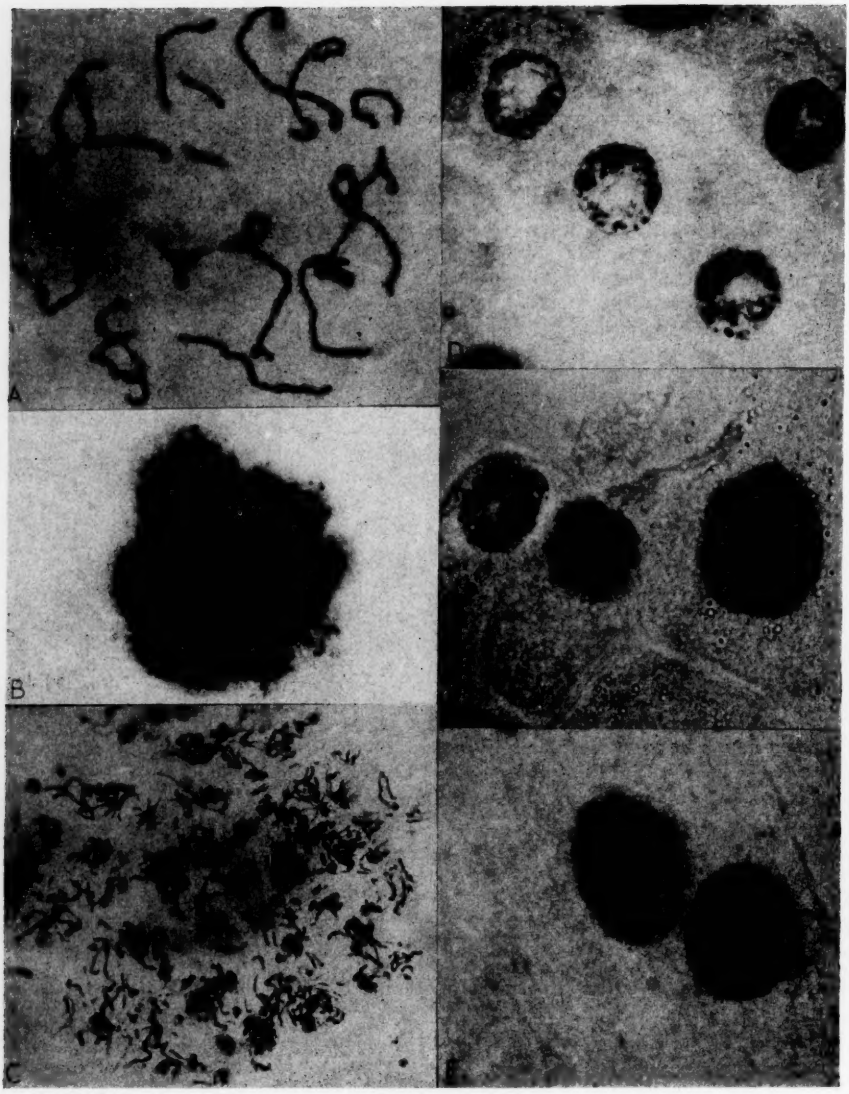

NUCLEI IN RHOEO ROOTS

Figure 1

A—Prophase from normal meristem (1300 ×); *B*—Energic nucleus from the same root as *C* (1100 ×). *C*—Prophase of meristem of root treated seven days with colchicine (550 ×). *D*—Energic nuclei from normal meristem (1100 ×). *E*—Energic nuclei from third millimeter section (1100×). *F*—Energic nuclei from fifth millimeter section (1100×). Compare nuclear size and the number of dark-staining heterochromatic bodies in *D*, *E*, and *F*.

THE NUCLEUS IN DIFFERENTIATION AND DEVELOPMENT

I. Heterochromatic Bodies in Energic Nuclei of Rhoeo Roots

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It seems probable that in differentiation and development the nucleus exerts its influence on the cytoplasm and reacts with substances therein predominantly while it is in the "resting" or energic stage. During the past twenty years of rapid developments in cytogenetics, however, comparatively little attention has been paid to the energic stage, particularly of plants.

One of the first things to be determined is obviously the chromosome constitution. Geitler[9] developed an indirect method for doing this in certain insects by the study of heterochromatic regions, but most of the studies of these in plants have been concerned only with the significance of "prochromosomes." They pre-date our present understanding of the nature of heterochromatin and therefore fail to give adequate information on certain issues now seen to be important. This is the first of a series of studies on chromosome conditions in differentiated tissues. It is concerned chiefly with the indirect determination of differences in chromosome number in energic nuclei of meristematic and differentiated tissues in the roots of *Rhoeo*.

The possible bearing that such a study may have on interpretation of gene action in development and differentiation has been discussed elsewhere[16,17]. Here, only some data directly related to the problem of the energic nucleus will be discussed. The various factors that have been considered as evidence for the interaction of the energic nucleus with the cytoplasm are: 1) the varied shapes and sizes of nuclei in tissues of the same organism (see Geitler's examples and others cited herein); 2) their different stainability, ascribed by earlier workers to oxy- and basi-chromatin, which can now be interpreted in terms of ribose and desoxyribose nucleic acids, (e.g., the generative and vegetative nuclei of the pollen grain in angiosperms); 3) the variation in chemical composition of nuclei of different tissues[25]; 4) the fact that the nucleus is found in the cell at the site of greatest activity, (for example there is movement of the nucleus to the site of local cell wall growth in plants[33]); 5) the variation in size and number of nucleoli (e.g., we find that the long phloem nuclei in *Rhoeo* roots always have two distinct, relatively small nucleoli, while the parenchyma cells have one large nucleolus); and 6) the maintenance of the nuclear-plasma ratio in cells of very different size (e.g., the uninucleate pollen-grain and anther wall cell in *Malva crispa*[8]). All these factors, though not direct evidence for the leadership of the energic nucleus, at least show that it plays an important role in the physiological activities of the cell.

Berrill and Huskins[2] have presented evidence of suspended physiological activity of cells undergoing mitosis, thus emphasizing that the energic nucleus is the physiologically active one. Stern[32] showed that the changed genetic constitution of a nucleus can directly, without intervening mitosis, determine the char-

*Working under a Fellowship of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

acter of its cell; his best examples are the unicellular twin spots in *Delphinium ajacis*[5] and the mating reaction of sister cells in the promycelium of *Ustilago*[18]. Hämmerling's[13] regeneration experiments in *Acetabularia* show clearly that a non-dividing nucleus may exert a determining influence on differentiation by establishing a gradient in the cytoplasm of its cell. Furthermore, even in the actively dividing plant meristem only one out of every eight nuclei is mitotic, while the other seven are interkinetic[12]; thus the relative durations alone clearly stress the importance of the energic phase. Most differentiation, as well as most metabolic activity, occurs in non-dividing cells, and continues long after mitotic activity has ceased; this makes it probable that stimuli for these activities are derived from the energic nucleus. An original stimulus could, however, be transmitted to the cytoplasm at the time of mitotic division and, once started, a cytoplasmic chain of reactions could continue without further influence from the nucleus.

Goldschmidt[11] interpreted the correlation of waves of mitosis with wing pattern in *Ephestia*[3] as evidence for the release of determining substances during mitosis. Since such waves of mitosis reflect previous chromosome reproduction during which determination could already have occurred, and since there is a time lag between the mitoses and the phenotypic appearance of the pattern, this correlation is not decisive evidence for release of substances in mitosis. Certainly Goldschmidt would agree that it is no evidence for their *production* during mitosis.

The observation, in the tissues of man by Jacobj[19] and of other mammals by G. Hertwig[15], of definite nuclear size classes that fit into a geometric progression, has been interpreted by them to mean that short periods of inner nuclear growth alternate with longer resting periods. This, of course, occurs without mitosis and is caused according to Hertwig by inner division of the chromosomes or endopolyploidization. Jacobj's measurements show a complete range from the smallest nuclei in the erythroblasts of the newly-born infant (size index ⅛; 1 being the most frequently occurring nuclear size) to the largest nuclei in the spinal ganglion cells (size index 32). In some tissues nuclei of several size classes are found. Each class is double the preceding one according to Jacobj's data, while G. Hertwig found intermediate classes where, according to his interpretation, a "physiological factor" enters in. This physiological factor can, he considers, cause a doubled nuclear surface and therefore more than a doubling of the volume. Other cases, where intermediate size classes are found and the volume is less than doubled, may be interpreted as due to maintenance of the nuclear-plasma ratio in large vacuolated cells[10].

By 1929 Heitz' work[14] on the heterochromatic bodies in interkinetic nuclei of *Pellia epiphylla* had shown that these bodies are identical with chromosomal regions in definite positions along the length of the mitotic chromosome. In prophase the euchromatic parts of the chromosomes appear always in the same relation to their heterochromatic segment, which in some cases becomes unstainable as the euchromatin becomes stainable. Conversely, in telophase the heterochromatic regions retain and the euchromatic parts of the chromosome gradually lose their stainability. Thus Heitz showed that the terms "prochromosome" and "chromocenter" alike imply an erroneous idea, namely that the mitotic chromosome condenses around a center which alone is maintained in the interkinetic nucleus. An agreement in number of heterochromatic bodies with the actual chromosome number can come about merely by coincidence or by each chromosome having one heterochromatic region. The latter is actually the case in some well-known plants, where the region near the kinomere consists of heterochromatin[6]. In such it may not be too unjustifiable to use the otherwise misleading term "prochromosomes."

In organisms with a countable number of heterochromatic bodies, examination

of energic nuclei in various tissues can give information on changes occurring during differentiation. Geitler[9] has carried out the most detailed study of such differentiated nuclei in many tissues of the water skater, *Gerris lateralis,* where the large distinctive-shaped sex chromosomes can be seen in all nuclei. Where they are few in number their frequency can be seen to agree with the number of sets of euchromatic chromosomes. Where the number of heterochromatic sex chromosomes is large the euchromatic chromosomes can no longer be seen, but Geitler justifiably assumes that their frequency can be taken as an indication of the degree of polyploidy of the nuclei of differentiated tissues. In the male, with only one X-chromosome, Geitler found 1,024 heterochromatic bodies in the much-lobed salivary gland nuclei, which indicates 2,048-ploidy; sections of the Malpighian tubules become 32-ploid, others 64-ploid. The testicular septa are octoploid. In the epithelial cells of the midgut the actively working cells, which are octoploid, are replaced after each digestive act from diploid reserve cells. Geitler convincingly showed that all these nuclei become polyploid by endomitosis, for in some nuclei the various phases of an inner nuclear division can be observed: the chromosomes become stainable, contract and pass through pro-, meta-, and anaphase, the difference from true mitosis being that the nuclear membrane does not disappear, no spindle is formed and anaphase movement to the poles is lacking.

In *Anthoscelides obtectus,* the bean weevil ($2n=20$), Bushnell[4] found that the midgut epithelial cells have nuclei of two size classes, the larger ones having twice as many "prochromosomes" ($4n=40$) as the small ones. At the beginning of each larval instar new functional epithelial cells appear to be formed from the smaller regeneration cells. During the feeding period they double in size. There are so many "prochromosomes" in polyploid nuclei that the exact determination of their number is difficult. Therefore, when Bushnell found a nucleus with 100, which would correspond to five times the diploid number, he believed this to be a faulty count. He considered, but rejected the possibility of a differential rate of reproduction of the chromosomes. We suggest, on the basis of our data on polyteny in differentiated plant tissues, that a decaploid nucleus could have arisen from chromosomes that have become five-stranded before separating into individual chromosomes. Nuclear fusion as the mode of origin of such polyploid nuclei, which has been suggested by numerous earlier workers[22], is believed improbable by Bushnell, Geitler and ourselves. It may be added that Roberts and Struckmeyer[26] emphasize the absence of evidence for nuclear fusion in plant graftage where it has so often been claimed. Neither Bushnell nor Geitler found somatic reduction of polyploidy, as Berger[1] found in *Culex pipiens.* In tissues where lower degrees of polyploidy occurred after higher ones, as in the midgut epithelium, they explain these by renewal from diploid nests of regeneration cells. Thus their implication is that nuclei which have become endopolyploid may be highly active in some metabolic processes, but as far as their ability to divide or regenerate new tissue is concerned they are at the end of the line and destined to degenerate.

Studies on "prochromosomes" in plants have yielded few data pertinent to the present issue. Doutreligne[6] found great variations in number but failed to state what tissues were studied. Rosenberg[27,28] and Lundegårdh[23] found less variability. For our purpose the issue is not variability *per se,* but whether there is great variability around a single mode or more limited variability around several modes. Kuhn,[21] who found agreement between the number of chromosomes and heterochromatic bodies in late telophase and energic nuclei respectively in *Thalictrum, Phaseolus* and *Capparis,* was deterred from assuming any significance for this by the facts that: a) in these plants the chromocenters disappear before earliest prophase; b) in older nuclei "secondary changes of chromo-

center number" occur. He concluded that the chromosomes neither arise from the chromocenters nor do they have any connection with them as Heitz had indicated. His observation that the number is reduced in cells of the root cap and the fact that his drawings show in some cases that decrease in number is accompanied by increase in size of chromocenters may, nevertheless, have significance for the present studies.

Manton[24] who found agreement of chromosome and prochromosome number in *2n*, *3n*, *4n*, *5n* and *6n* plants of *Biscutella laevigata* also found cases of polysomaty in it and several other plants, but ascribed them to the occurrence of sectorial chimaeras. One case, in *Iberis semperflorens*, appears clearly to be a chimaera but it is possible that others could be polyploid cells, rather than whole sectors.

Methods and Observations

Cuttings of *Rhoeo discolor* (*n*=6) were rooted and grown in a modified Hoagland's solution in dark glass bottles of 450 cc each, in which they were inserted through holes bored in paraffined corks. The roots were thus kept in the dark while the plants were exposed to full light in the greenhouse. The culture solution was kept at pH 5.7 and its temperature was maintained at 20°C ± 2°C by immersing the bottles in a water bath during warm weather; in these ways the environmental conditions were partially controlled.

Roots were fixed in alcohol-acetic acid (3:1) and stained by the Feulgen method. After staining *in toto*, the root, starting at the tip, was cut into five 1 mm sections, each of which was smeared separately. Slides were made permanent by floating off the coverslips in 95% alcohol and mounting in diaphane or by dehydrating in dioxane and mounting in balsam. Some roots were also fixed in chrom-acetic-formalin (Craf) embedded in paraffin and sectioned at 14 micra. They were stained by the Feulgen method and counterstained with Fast Green.

In smear preparations the number of heterochromatic bodies in the meristematic region was found to average 10.6, varying from 6 to 19; the most frequent numbers found were 10, 11, and 12. (Table II, Figure 1*D*.)

To ascertain whether the number of heterochromatic bodies in interkinetic nuclei was a good indication of the number of chromosomes in such nuclei a *Rhoeo* plant was grown for one week in culture solution to which 1% colchicine had been added. Preparations made from fixations at various times during this week showed that the chromosome number of meristematic cells was doubled once every 24 hours. All phases of mitosis or their equivalents were observed. However, the daughter chromosomes did not move to the poles and thus after each anaphase separation all were included in the same telophase nucleus. A very high degree of polyploidy resulted after one week. Figure 1*A* shows a prophase from an untreated meristematic cell for comparison with Figure 1*C* from the seven day colchicine treatment. This prophase and the vesicular resting nucleus (Figure 1*B*) are estimated to be 256-ploid, but accurate counts of such high numbers are impossible. Roots in which *4n* and *8n* metaphases were found contained energic nuclei with 24 and 48 heterochromatic bodies of the same size as in untreated

TABLE I. Heterochromatic Bodies in Energic Nuclei of *Rhoeo discolor* Roots. (Sectioned preparations.)

Region	No. Nuclei	Av. No. Het. B.	Variance	S. D.
Meristem	100	8.0	5.29	2.3
Differentiating	60	8.5	3.24	1.8
Differentiated	68	13.5	21.4	4.63

TABLE II. Number of Energic Nuclei Containing Various Numbers of Heterochromatic Bodies in Roots of *Rhoeo discolor*. (Smeared preparations.)

Region of root	No. of heterochromatic bodies 5-9	10-14	15-19	20-24	25-29	30-34	35-39	Mean
1 mm	39	138	13					10.62±2.5
2 mm	17	66	17					12.0 ±2.9
3 mm	4	22	34	29	10			17.9 ±5.2
4 mm		11	30	40	18	1		20.4 ±4.6
5 mm		8	31	36	21	3	2	21.3 ±5.56

Differences between means:

1 : 2 not significant
1 : 3 not significant
1 : 4 9.78 ± 3.46 P = 0.005
1 : 5 10.68 ± 4.12 P = 0.01

meristems. This is the number expected if the number of heterochromatic bodies corresponds to the chromosome number. In sectioned preparations the number of heterochromatic bodies in energic nuclei both in the meristematic and in the differentiated regions was much lower than in smeared material (Table I). The trends, however, are the same in both. The discrepancy can be attributed to the method of fixation and preparation. It is obviously much easier to get an accurate count in swollen, flattened nuclei of smear preparations; heterochromatic bodies lying close together will frequently be separated sufficiently to distinguish them in such preparations, and those actually fused will more clearly exhibit their compound nature. For these reasons the counts made on smear preparations are regarded as more reliable, and the data from sectioned roots will not be considered further.

Table II shows the number of heterochromatic regions in energic nuclei in smear preparations in different millimeters of the root. The first millimeter is always entirely meristematic; the second millimeter may contain some meristematic and some differentiating cells; the third millimeter is usually still in the process of differentiation and the fourth and fifth millimeter are distinctly differentiated in that long phloem cells and annular vessels are present. In making counts it was realized that a highly subjective element is hard to exclude. The unconscious selection of nuclei to be analysed would tend toward those with fewer sharply stained regions. The long nuclei of phloem elements were consciously eliminated since they in themselves might give a different picture. Large heterochromatic clumps that were clearly formed by fusion of several small regions were recorded as the number of small regions into which they could be resolved.

It can be seen from Table II that the number of heterochromatic bodies increases in the nuclei of differentiated tissues. The correlation between the distance of a nucleus from the tip and its number of heterochromatic regions is $r = 0.6$; this is highly significant.

The graph, Figure 2, shows that in the meristematic region (first and second mm) there is a distinct mode at 10-14. In the distinctly differentiated region (fourth and fifth mm) the mode is at 20 - 24 but there is a very wide scatter. In the third millimeter, in which differentiation is in active progress, the scatter is so great that there is no clear mode. This may at first seem surprising, for we are accustomed to think of diploid and polyploid as sharp alternatives and therefore might expect the nuclei to have either twelve or a multiple of twelve heterochromatic bodies if these are correlated with the number of chromosomes. The polyploidy of differentiated tissues, however, arises through endomitosis, and, as will be shown in the succeeding paper, polyteny is an initial stage in the process, at least in *Rhoeo* and barley. When a chromosome is becoming polytene, it remains unitary longest in the region of the kinomere, with which the heterochromatic regions are associated in *Rhoeo*. Separation at the kinomere is a gradual process. It is therefore not surprising that in many nuclei the number of distinct heterochromatic bodies is intermediate between the two modes. The frequency of intermediate numbers may be an indication of the frequency or rate with which endomitosis is occurring.

The trend of increased number of heterochromatic bodies in nuclei farther away from the tip is so definite that the differences between the first and fourth and the first and fifth millimeter are statistically significant, Table II.

In the roots given continuous treatment with colchicine the number of heterochromatic bodies in energic nuclei was in many cases similar to the number of chromosomes in nearby dividing nuclei. If the same is true in these untreated roots and if numbers of heterochromatic bodies intermediate between multiples of twelve indicate nuclei that are polytene or undergoing endomitosis, then in the fourth and fifth millimeter

more than a third of the nuclei are tetraploid, almost a third are becoming tetraploid and slightly more than a quarter are becoming octoploid. Only one tenth have remained diploid. The presence in the meristematic region of nuclei with fewer than twelve heterochromatic bodies shows, however, the limitations of the data and of this argument. To explain them we have to assume either: a) fusion of heterochromatic bodies, which is known to occur, or b) that they are in nuclei becoming specialized to form root-cap cells. In the root cap nuclei the mean number of heterochromatic bodies is only 4.6. The significance of this is not known, but Kuhn[21] found a similar decrease and Rosenberg[28] found variation in size and number in the tentacles of *Drosera* after experimental feeding.

Similarly we must not assume in advance that the increase in number in differentiated regions is necessarily associated with multiplication of the entire chromosome complement, since it has been shown that heterochromatin may reproduce at a different rate from euchromatin in *Drosophila*[31] and *Paphiopedalum*[7]. This possibility is rendered unlikely, however, by the parallelism with different degrees of polyploidy in the colchicine experiment mentioned above and perhaps by the fact that there is a clear association between number of heterochromatic bodies and nuclear size, Figure 1*D*, *E*, *F*. That the simplest explanation, i.e. that increase in number of heterochromatic bodies is due to polyploidy, is almost surely the correct one will be shown in the succeeding paper.[17a] In it experiments are reported on the stimulation to division of nuclei in differentiated cells, most of which would not otherwise have divided again.

In *Trillium sessile* ($2n = 10$) energic nuclei from meristematic and differentiated regions of the root and from differentiated leaf tissue are strikingly similar in number and size of heterochromatic bodies. Nuclei of various shapes, however, are found in the differentiated tissues. In almost all energic nuclei there are seven heterochromatic bodies. These are of three size classes; usually there are two large ones, four of medium size, and one small one. In the meristem two to four nucleoli are found while in differentiated tissue, where they are rather difficult to see, there is frequently only one nucleolus but sometimes as many as five. These data are reported here to emphasize that though in *Rhoeo* differentiation appears to be correlated with increase in the number of heterochromatic bodies, similar differentiation may occur in another plant without increase in their number. Preliminary observations indicate, however, that there may be an increase in the degree of polyteny in some differentiated cells of *Trillium*.

Discussion

The present study shows, as did the earlier studies of "prochromosomes" in plants, that there is no simple 1:1 relationship between the number of heterochromatic bodies seen in energic nuclei and the number of chromosomes at mitosis. There is a direct relationship but it is obscured 1) by the frequent fusion of heterochromatic bodies; 2) by the variation in their size, apart from fusion, through what may be classed as physiological factors; 3) by variations in staining capacity, and other reactions involved in the making of the preparations. To these must be added the subjective factors which play a part in every cytological study but particularly in one dealing with structures that are in themselves variable in their manifestations. However, when all discounts are made it remains obvious that the number of heterochromatic bodies gives some indication of the number of chromosomes present and this is significantly greater in the differentiated region of the fourth and fifth mm. from the root tip than in the meristematic region of the first and second mm. The modal number is approximately double, but the variability is very great.

This variability parallels that of nuclear size which has been extensively

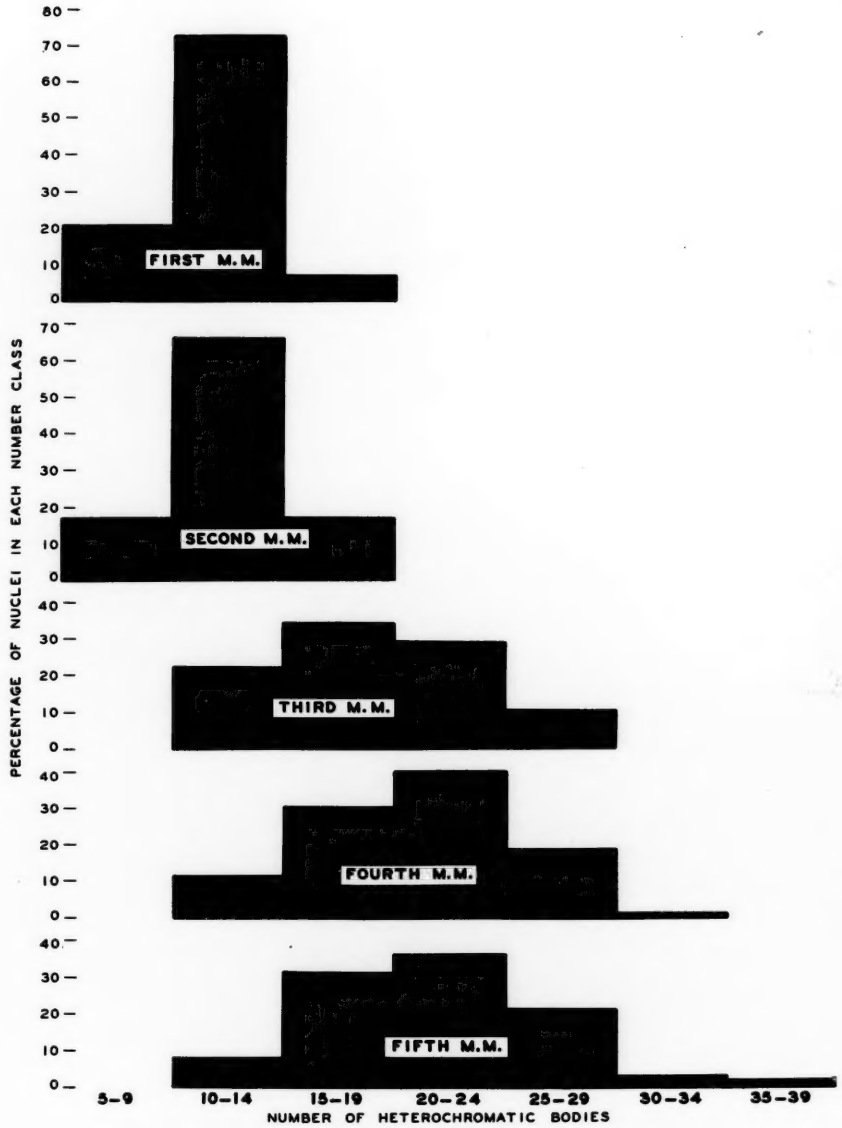

Figure 2

Number of heterochromatic bodies in consecutive one-millimeter sections of *Rhoeo* root-tips. The first millimeter contains almost exclusively meristematic tissue. The cells farther back in the root are in increasing proportion differentiated, though not yet highly specialized. The mean number of heterochromatic bodies per nucleus in the fifth millimeter section is approximately double that in the first millimeter section.

analyzed in mammalian tissues, including human, and also in fish[30]. That nuclear size can be affected enormously by physiological and other factors apart from any change in chromosome constitution has been abundantly demonstrated under both natural and experimental conditions. That reported doubling of the chromosome contents also plays an important part in the production of the variability in nuclear size seemed equally evident to Jacobj[19] and to others from the fact that the curve of variability was multimodal with each successive mode approximately doubling its predecessor. The correctness of this conclusion could not be tested directly, but the mass of indirect evidence now available from other organisms, especially insects, has made it appear almost self-evident.

In succeeding studies of this series it will be shown that increase in chromosome number in the differentiated tissue of *Rhoeo* and barley roots comes about through successive reproductions during the energic stage and by endomitosis, not by a "colchicine-effect," i.e., through abortive mitoses and the formation of "restitution nuclei." This gives a ready explanation for the intermediate numbers of heterochromatic bodies, especially when, as in *Rhoeo,* they occur in association with the kinomere. In endomitosis the division of the multi-stranded "polytene" chromosomes into separate chromosomes does not occur synchronously and suddenly in all of them as it does at anaphase of mitosis. The region of the kinomere is the last, instead of the first, to undergo permanent separation. Instead of abrupt doublings of the chromosome number as in mitosis, there is therefore a gradual change from diploidy to tetraploidy, from tetraploidy to octoploidy and so on. If this occurs during the energic stage also, as indirect evidence indicates, the gradualness of the change will be most evident for the heterochromatic bodies.

The regular occurrence of polyploidy in certain insect tissues has led most cytologists to conclude that it must have some relationship to differentiation and/or physiological function. The latter was stressed when it appeared that polyploidy or polyteny was most frequent in gland cells. With increasing evidence of its wide-spread occurrence in many tissues some wider interpretation of its significance has become necessary. It has been suggested[16] that polyteny, and in turn polyploidy, is a reflection of continuing reproduction during the energic stages of the fundamental sub-units ("lamellae") of which it is provisionally assumed the gene of heredity is made up. From such a basic postulate, working hypotheses can be constructed to direct experiments or examine data on the role of the nucleus during differentiation and development. It cannot, of course, be assumed that polyteny, or polyploidy, in itself directly determines differentiation. Similarly, it cannot be assumed that if, as suggested, polyteny is a reflection of gene reproduction it should always be present. In the first place there must, on the hypothesis, be many reproductions of the "lamellae" before chromosome strands thick enough to be seen under the microscope can be produced. Secondly, if the postulated reproduction of the lamellae is the means by which the genes act in differentiation and development, then either the lamellae themselves must be the "gene products" and as such be used up, or in their reproduction by-products arise which have that function. In either case the chromosome of the energic nucleus must be looked upon as a structure in dynamic equilibrium which can be increased or diminished in size, and probably in its "strandedness"[20, 29], according to its physiological condition and function.

In some organisms and some tissues changes in the nucleolus accompany differentiation. In *Rhoeo* multiplication of heterochromatic bodies which indicates multiplication of chromosomes occurs in the fourth and fifth mm. of the root, while in its root cap there is reduction in the number of heterochromatic bodies, which almost surely does not indicate reduction in chromosome number. In many insect tissues there are uniform degrees of polyploidy while others have varying numbers, i.e. they are "mixo-

ploid." In yet other insect tissues the chromosomes are composed of a relatively small number of strands of relatively great diameter, i.e. they have a readily determinable but low degree of polyteny, while in the salivary glands of the Diptera they are fused in pairs and are almost surely polytene to a very high, and varying, degree but the strands are so fine and/or so fused that direct determination of their polytene nature is difficult if not impossible. In maize endosperm the chromosomes of the older nuclei are polytene, many being obviously 8-stranded and others even more multiple, (Duncan, unpublished). There does not appear to be a corresponding increase in the number of heterochromatic bodies. In *Trillium sessile,* as mentioned above, there are differences in the nucleoli but not in the number of heterochromatic bodies and there is probably variation in degree of polyteny. It is hoped that from some hypothesis such as that we are currently guided by[16] it may be possible to unite all these diverse cytological correlatives of differentiation into a functionally significant relationship. If not, the fact that polyteny (and polyploidy) so commonly accompanies differentiation, as herein indicated for *Rhoeo,* demands that some other rationale for it be sought. The data from organisms of many diverse types are now too numerous to be left piled up without any serious attempt being made to understand their significance.

Summary

In *Rhoeo discolor* there is a significant increase in the number of heterochromatic bodies in the differentiated, though not highly specialized, cells of the fourth and fifth millimeters from the tip. This is attributed to endomitosis and thus related to polyteny and polysomaty. In turn, these are related herein to a working hypothesis on gene structure and action, presented elsewhere, in which an attempt is made to correlate microscopically visible differences in energic nuclei with the processes of development and differentiation.

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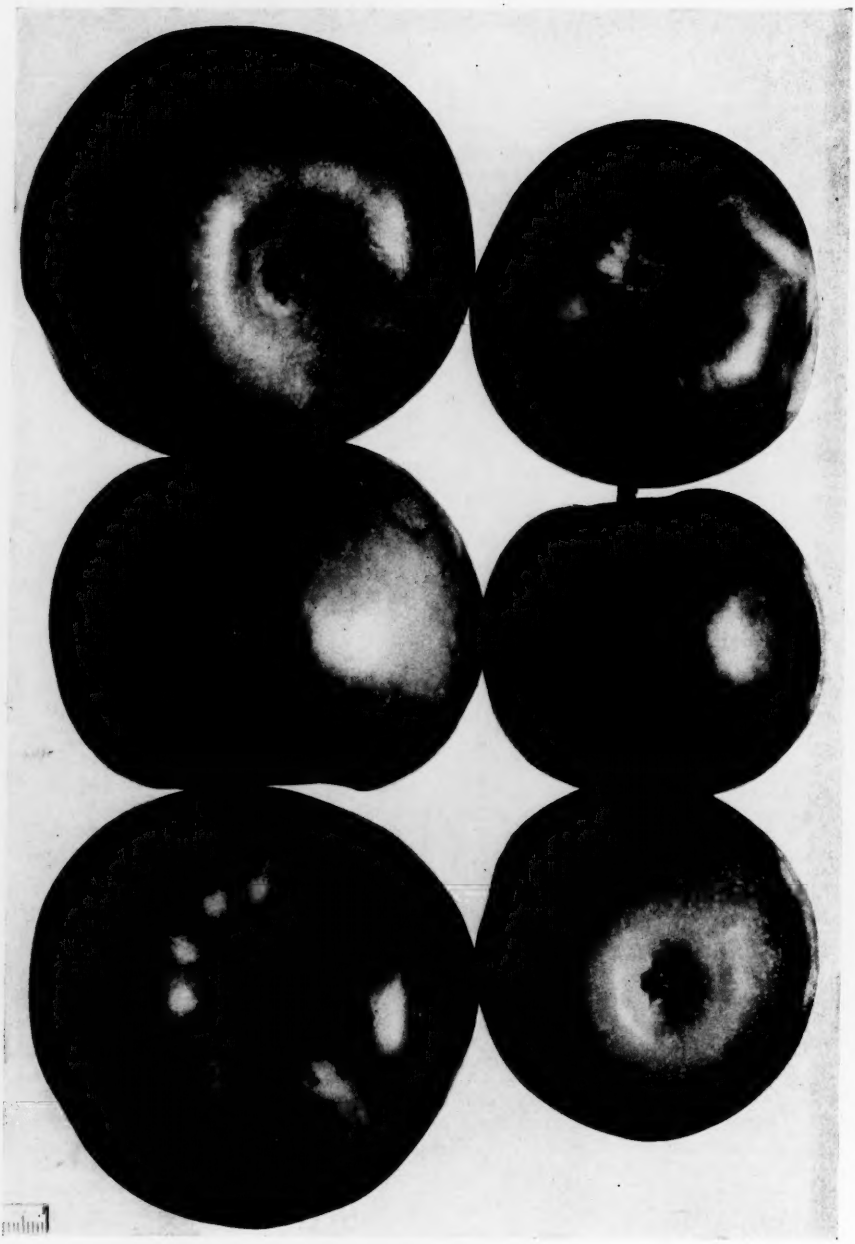

McINTOSH GIANTS

Figure 3

The Giant McIntosh apples are about twice as heavy, and a third larger in diameter, as the normal McIntosh. This "giant" strain is due to the tetraploid condition of all the tissues except the epidermis, which is diploid. (Millimeter scale on lower left.)

THE NATURE OF GIANT APPLE SPORTS

And Their Use in Breeding

GEORGE M. DARROW, ROY A. GIBSON, WALTER E. TOENJES AND HAIG DERMEN*

APPLE sports may be grouped in several different categories. Those affecting chiefly the color or size of fruit may be distinguished from those chiefly affecting the physiology of the fruit or tree, as early or late maturity, taste and texture of fruit, tree growth and hardiness. Some color sports have proved of great economic value, not only because of the market appeal of highly colored fruit, but also because they have simplified some of the problems of the grower, such as the production of fruit of high color even under adverse conditions. Russet sports seem not as yet to have been of any value.

Giant sports have heretofore mostly been mere curiosities, for the trees are quite often unproductive and the fruit usually irregular in shape. The fruits of the giant sports have also had the reputation of being coarse-grained, and earlier-maturing than normal. However, in the instances recently discovered giant sports may prove to be of direct horticultural value. A "giant" Wealthy from Massachusetts is reported to be larger than normal, to have flesh of fine texture, to tend to overbear less severely than the normal and to be more consistently an annual bearer than the normal. A "giant" Ontario sport seems to bear a fairly adequate crop.

Extent of Polyploidy

The greatest value of some "giant" sports, however, may lie in their use in breeding for the mass production of triploid, tetraploid, pentaploid, and hexaploid apples, from which selections for desirable seedlings could be made and on a large scale.

The development of polyploid strains through the use of giant $4x$ sports seemed such a promising possibility that a survey was made of the literature to learn of the material available for such use. This is listed below:

(1) On the basis of pollen size, two "large-fruit" Bartlett pears were reported by Cartledge, Shamel, and Blakeslee[2] to be $4x$. The second histogenic layer and possibly the third was also tetraploid. The Karr sport bore fruit up to twice normal size, with larger than normal leaves and flowers, and when propagated by Mr. Karr in 1924, reproduced its large fruit size.[12]

(2) On the basis of pollen size, the Parbetron, a giant Bartlett sport, has been found by Dermen (unpublished data) to be $4x$ at least in the second histogenic layer. Its pollen grains and egg cells are $2x$, and it has been used in breeding.

(3) Olmo in 1942[11] reported that the "giants" of grape varieties were tetraploid. He had 20 tetraploid *vinifera* varieties as well as tetraploids of Campbell, Catawba, Delaware, and Niagara. He found the effects of polyploidy to be variable. The $4x$ Niagara produced well, its larger berries offsetting the scantier set of fruit. Catawba had very irregular clusters. Although most $4x$ *vinifera* varieties had a poor growth habit and poorly filled clusters, the $4x$ Carignane produced well filled clusters. The $4x$ Muscat of Alexandria was actually more fertile than its diploid. Eight tetraploid varieties (one *vinifera* and seven American or hybrid) have been introduced and some of them have been grown commercially. In three tetraploid seed-

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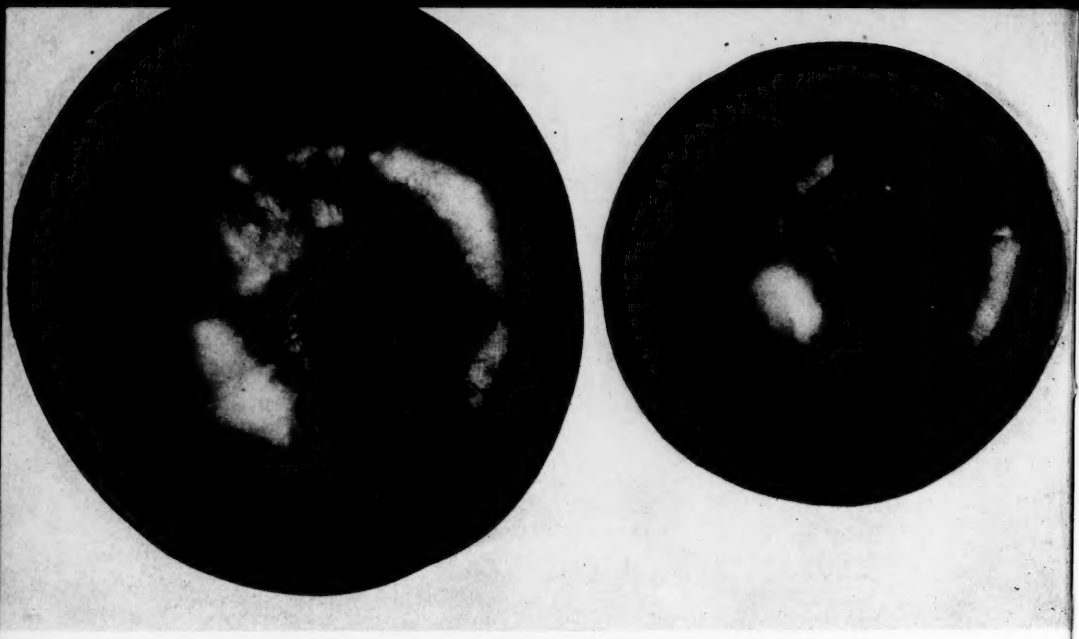

NORMAL AND GIANT McINTOSH—NATURAL SIZE

Figure 4

In quality and texture the "giant" form is fully equal to the normal, though it may not keep as well. In yield it compares very unfavorably with the normal. Mass selection of tetraploid seedlings offers great promise that superior varieties may be produced by this technique.

ings the giant berry size was maintained. In one it exceeded the tetraploid parents.

(4) Dermen and Bain[7] obtained by colchicine technique tetraploids of all the well-known cranberry varieties. These plants had various degrees of ploidy in different tissues. Where the second histogenic layer was tetraploid the pollen grains and egg cells were $2x$. Some of these types have been crossed together to obtain progeny at the tetraploid level (unpublished data). When the third layer only was tetraploid the pollen grains and egg cells were normal haploid ($1x$).

(5) Dermen[4] succeeded in producing tetraploids of pear varieties. He was able to obtain triploids by crossing these with diploids. He has also reported obtaining tetraploid peaches by colchicine technique.[3]

(6) Einset and associates[8] found the "giant" Wealthy apple to be $4x$ in all but the epidermis; and so it can be used in breeding on the polyploid level. Blaser and Einset's[1] evidence supports the assumption that the "giant" Northern Spy, Jonathan, and Rome Beauty sports have an innermost, third histogenic of $4x$ tissue: the first and second layers are $2x$. They therefore would not transmit polyploidy if used in breeding.

Giant apple sports so far studied fall into two general types described by Einset and associates. (1) Those like the "giant" Northern Spy, have only the third histogenic layer tetraploid and produce haploid pollen. (2) Those like the Wealthy, have all internal tissues tetraploid and a diploid epidermis. These "giant" apples seem to follow the pat-

TABLE I. Survey of apple sports at Graham Experiment Station

Variety	Orchard number	Description	Flowers
1. Baldwin	376	"Giant-fruited"	Normal
2. Delicious	384	"	"
3. Fameuse	247, 499	"	"
4. Graham (Spy)	775-780	Large-fruited	"
5. Grimes	377	"Giant-fruited"	"
6. McIntosh	134, 583	"	"
7. Northern Spy	570	"	"
8. Oldenburg (Duchess)	398	"	"
9. Ontario	39T	Solid red	Large flowers and pollen grains*
10. Rhode Island	553	Large and small	Normal
11. Steele Red	526-532 587-592	"Giant-fruited"	"
12. Wealthy	124	"	"

*Pollen grains of Ontario appeared to be as large as those of Hibernal, which is known to be $4x$ and $3x$.

tern of the cyto-chimeral tetraploid "sports" induced in the cranberry. The evidence at present available supports the hypothesis that the giant apple sports are all chimeral tetraploids.

At the Graham Branch Experiment Station at Grand Rapids, Michigan, Roy Gibson and V. R. Gardner have collected many naturally occurring sports of apples and other fruits. Many of these are recorded as giant-fruited. In May 1947 these were examined visually to see if the flowers showed any evidence of being tetraploid and thus of possible use in breeding, and to obtain information on possible tetraploid characteristics. For the most part the trees were full of flower clusters, seemingly as full as normal trees of the same varieties. The varieties listed in Table I were examined.

A great many flowers were examined on many trees; except for Ontario they were normal and the pollen grains examined were of normal size. All flowers of Ontario examined were larger in all their parts and had larger pollen grains than the other varieties. The difference in size of flowers was great enough so that if many of those on the "giant" trees had shown comparable size-differences this would have been noted.

A second examination in September showed "giant" fruits on McIntosh, Northern Spy, 210-A (a Bismarck type), Ontario, Delicious, and Baldwin, as well as abnormal-shaped, possibly giant fruits on Rhode Island Greening and Grimes Golden. Large fruits, mostly normal in shape, were found on sports of Steele Red and Jonathan. All the Oldenburg (Duchess) fruit appeared normal. The giant Wealthy tree had no fruit. As might be expected from Dermen's experiments with cranberries, the trees and fruit would not be entirely "giant", but would have diploid and tetraploid tissues periclinally or mericlinally combined. Only by following out branches having the most fully giant fruits can buds and propagations containing entirely $4x$ internal layers be isolated.

In the light of this concept, the Ontario trees would be expected to be almost or wholly "giant" with both second and third histogenic layers probably tetraploid. The McIntosh and 210-A trees also seemed to be mostly of "giant" constitution, with the third layer $4x$. In the Baldwin, Delicious, and Northern Spy trees only certain branches were found to be of "giant" type. The Jonathan and Steele Red trees require further study. They may be in part "giants," or the increase in size may be due to genic rather than to chromosomal differences. The Rhode Island Greening and Grimes Golden trees, have sectors of "negative giantism," with fruits smaller than normal.

The trees of the "giant" sports have a distinctly different appearance, noticeable especially in the McIntosh and Ontario sports: a very spreading habit due to wide-angled branching. The trees of McIntosh in addition are dwarfed, but no other consistent difference could be seen in tree or foliage characters. In most of the giant trees there were some normal-appearing branches. A characteristic of some giant fruits is their flattened or oblate shape, very noticeable in Ontario, McIntosh, and Northern Spy, with less flattening in Baldwin and Delicious, and no flattening in the "large" Jonathan and Steele Red. When all these sports are examined cyto-histologically the nature of their increase in fruit size will be more accurately determined. (See Figures 5 and 6.)

In "rogue" type fruit, such as that of some Grimes Golden and Rhode Island Greening trees, where the irregular fruit is mostly smaller than normal, a gene mutation may be involved.

Typical comparable "giant," "large," normal, and "small" fruits, insofar as obtainable, were weighed and the results are given in Table II.

Except in the case of Ontario, the normal fruits of which came from Canada, the comparisons are between giant and normal fruit grown under similar conditions. Excluding Ontario, the fruit of the "giants" ranged from 49 per cent

WIDE-SPREADING "GIANT" TREES
Figure 5

To left, normal McIntosh; to right, "giant"-fruited McIntosh having a wide-angled, nearly horizontal branching habit in contrast to the acute-angled erect-growing habit of the normal McIntosh. (Graham Station, Michigan.)

TABLE II. Comparison of weight of giant, large, normal, and small fruits of some apple varieties.

Variety	Type	Number fruits	Weight (lbs.)	Percent of normal	Remarks
Baldwin					
No. 120	Giant	20	10.4	208	Relatively smooth
"	Normal	20	5.0	----	----
Delicious	Giant	20	11.8	184	Relatively irreg.
"	Normal	20	6.4	----	----
Northern Spy					
No. 510	Giant	20	12.8	149	Very irregular
"	Normal	20	8.6	----	
Ontario	Giant red	20	10.3	----	Relatively smooth
" (from Ontario)	Normal	20	8.6	130	
McIntosh	Giant	20	10.5	210	Relatively smooth
"	Normal	20	5.0		
"	Small	20	2.7	54	Elongated
" (from Mass.*)	Giant	12	11.5	198	Relatively smooth
" (from Mass.*)	Normal	12	5.8	----	----
Jonathan	"Large"	20	7.2	144	Relatively smooth
"	Normal	20	5.0	----	
"	Small	20	2.2	44	Normal shape
Steele Red	"Large"	20	7.5	150	Relatively smooth
"	Normal	20	5.0	----	----
210-A (Bismarck)	Giant	20	11.0	183	Relatively smooth
"	Normal	20	6.0	----	----

*Fruit from Roger Kimball, Littleton, Mass., received through W. H. Thies. See Figure 3.

to 110 per cent heavier than the comparable normal, while that of the "large" sports was from 44 to 50 per cent heavier than the normal.

A comparative test of fruit quality was made on November 14. This indicated that the fruit of the "giant" Delicious was riper and more mealy than that of the normal Delicious. The "giant" or "large" sports of the McIntosh, Baldwin, Northern Spy, 210-A, Jonathan,

TABLE III. Comparative yields of giant and normal McIntosh apples from three sets of trees of the same age, Graham Station. The yields are the summed totals (in pounds of fruit) for the trees in each block.

Block No.	Giant	Normal	G:N ratio	Remarks
A-134	1,157			Summed 3-year yield, 3 trees in each block
A-172		2,572	45%	
B-583	488			Summed 4-year yield, 4 trees in each block
B-245		3,012	16%	
B-431		3,989	12%	
C-374	507			Summed 4-year yield, 3 trees in each block
C-408		1,776	28%	
C-410		2,280	22%	

DWARF GROWTH OF "GIANT" TREES

Figure 6

Normal McIntosh apple tree in foreground to extreme left, "giant"-fruited McIntosh to right. Note spreading top of the giant-fruited sport. (Graham Station, Michigan.)

and Steele Red were fully as good in quality (including texture) as the normal. The "giant" Delicious, with a small crop of giant apples, may have matured earlier than the normal to give the different character of flesh, and accordingly should have been picked earlier than normal. Under conditions of commercial storage the giant sports have been observed to break down earlier than the normal fruit.

The "small" McIntosh and Jonathan fruits weighed only 44 per cent and 54 per cent as much as the normal. The "small-fruited" trees of these varieties bore good crops but were erect-growing with slender branches. All the fruit was relatively uniform on several "small-fruited" trees of each variety.

Yield records of three comparable sets of McIntosh trees are given in Table III.

In all five comparisons in the three blocks, where there were comparable trees of the same age, the "normal" trees outyielded the "giant" trees; and in four of the comparisons they yielded two to several times as much.

Since we do not know the true nature of giant forms, the comparison of yields should not be taken too seriously. Possibly autotetraploids of some varieties are less productive than diploids. But the numbers studied are small. Some may prove to be fully as productive, better annual bearers, stronger in tree, and better adapted to particular environmental conditions. If this does not prove to be the case, the tetraploids may still have great value in breeding. Crosses of tetraploids may not display the shortcomings of the autotetraploid forms, and may be just as productive as diploids and with the average size of fruit much larger. At present we lack the information to draw definite conclusions.

Discussion

In order to appraise their value and utility, all these giant apple sports require intensive cyto-histological study.

It is very probable, on the basis of pollen-grain size, that the "giant" Ontario sport can be used as a tetraploid in breeding. It is quite certain, on the other hand, that the Northern Spy, McIntosh,* and Delicious sports cannot be so used. In the case of the 210-A, the Steele Red, and the Jonathan, further examination may reveal tetraploidy in either the second or the third histogenic layers or both, in some branches.

This preliminary survey indicates the need of a country-wide search for "giant sports" to locate, if possible, sports in other varieties having a tetraploid second histogenic layer, so that they can be used in the production of mass polyploid forms of McIntosh, Jonathan and Red Delicious varieties which have proved to be valuable as parents at the diploid level, need to be found and isolated. Next, we need to extend the use of methods such as those suggested by Dermen[5,6] for obtaining from trees that are tetraploid in the second and third layers, or in the third layer only, trees with all histogenic layers tetraploid.

Reports as to the quality and fruiting characteristics of the giant sports have been rather conflicting. This may in part be due to a failure to differentiate "giant" forms tetraploid in both the second and third histogenic layers from those with only the third layer tetraploid. The trees of "giant" Ontario had good crops of large apples in 1947. There were no comparable normal diploid trees at the Graham Station for comparison. Trees of the "giant" Wealthy have been reported to have borne good crops of large apples. For such young trees, the "giant" 210-A produced a fair crop. In contrast, the "giant" McIntosh had a small crop, far less than did the normal McIntosh. The other giants seemed much less productive than the normals. Just as with other fruits, we need seedling progenies before we can judge the value of tetraploid apples in comparison with diploids. We do know that some of the best varieties we now have, among them the Stayman Winesap, Baldwin, Rhode Island Greening, and Gravenstein, are triploids. The mass production of triploid seedlings by making diploid $\times$ tetraploid crosses, as well as tetraploid seedlings ($4x \times 4x$) is obviously a very promising method which needs to be explored.

The value of tetraploid apples in breeding has been discussed to some extent by Einset[8] and by Nilsson[10]. It should be pointed out that: (1) Tetraploids and hexaploids may be self-fertile, which makes provision for cross-pollination of self incompatible clones unnecessary and permits selfing in breeding. (2) Breeding at the tetraploid level between varieties known to be good parents at the diploid level would probably result in a higher percentage of desirable plants in respect to many characters than would crossing at the diploid level. Once tetraploid forms of known varieties are available for breeding, we should be able to obtain triploid or tetraploid seedlings in quantity so that we could undertake mass selection from large populations. Obviously it would be a much more rapid and effective procedure to obtain genetically better seedlings than to wait for the rare appearance of chance triploid or tetraploid seedlings.

Availability of tetraploids of named varieties should greatly stimulate breeding of tree fruits. In regions where hardiness of tree is needed and the quality of McIntosh is desirable, crosses between a tetraploid McIntosh and certain diploids, such as the $2x$ Delicious, may give very valuable new types. In even colder regions, where existing adapted varieties have very small fruit, this technique may combine extreme hardiness with larger fruit size. Instead of the existing small-fruited Printosh, apples of much larger size might be obtained by crossing the Prince with giant McIntosh, rather than with the normal diploid form.

This very preliminary exploration of

*A "giant" sport of McIntosh from Massachusetts was reported by Dermen and Darrow in the January issue of the JOURNAL OF HEREDITY to be internally tetraploid and epidermally diploid. This sport can now be used on the polyploid level.

the possibilities of polyploid apple breeding will have served a very useful purpose if it stimulates a wider interest in the application of new knowledge of chromosome constitution to the production of superior fruit varieties.

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✦

STATISTICAL COOKBOOKERY

THIS little book* is a guide to modern statistical methods, including the design of experiments, intended for those engaged in pilot-plant and plant-scale experiments in chemical industry, whence all its many examples are drawn. It claims to require no mathematics beyond arithmetic; therefore it is a statistical cookbook. Because of the lengthy discussion of the analysis of variance and of the factorial scheme of experimentation, including confounding, it will be of considerable value to research workers generally. For the geneticict, however, it cannot compete with books such as Mather's *Statistical Methods in Biology,* because so little space is given to the statistics of attributes (i.e., chi-square and the binomial and Poisson distributions). In addition to the topics already mentioned, there are chapters on heterogeneity of variances, the quality control chart, correlation and regression, multiple regression, balanced incomplete block designs, and general conclusions, and there are the usual tables of significance levels. There is no index, but the table of contents gives a title for each section of each chapter.

The book has several important technical defects. It neither makes clearly nor maintains the fundamental distinction between sample and parent population, nor is the essential importance of randomization in experimental design effectively emphasized. In tests of significance in the analysis of variance, Brownlee habitually uses high-order interactions as estimates of "error"; unfortunately the discussion of this practice is vague and inconclusive, and there is nowhere a plain statement of the desirability of experimental designs which yield estimates of "pure error." In addition, he recommends and employs the practice of testing the smallest "effect" variance first, pooling it with the test variance if nonsignificant, proceeding to test the next smallest, and so on. This scheme should be avoided. Pooling always raises awkward questions, not yet answered, as to the meaning of the subsequent tests, but the arbitrary selection of the smallest

(*Continued on page* 53)

**Industrial Experimentation,* by K. A. Brownlee, Chemical Publishing Co., Brooklyn, 1947, 151 pp.

This review should not be construed as representing the official position of the U. S. Atomic Energy Commission.

A DOUBLE SPLIT STERNUM IN A WHITE LEGHORN COCKEREL

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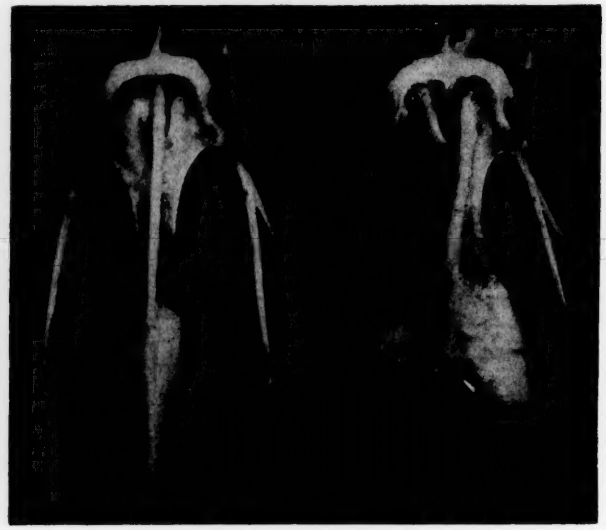

BIFID BREASTBONE
Figure 7
Normal sternum on the left, compared with the unique deviation found in a single cockerell.

CERTAIN experiments in progress at the Kentucky Agricultural Experiment Station concern comparative developments of the skeleton of six breeds of chickens.* These experiments require physical examinations of individual chickens at particular times during the period of growth.

One of the authors (MacLaury) while making such examinations found a White Leghorn cockerel which had an abnormally developed meta-sternum or caudal medial projection of the sternum. The cockerel was ten weeks old at the time, and weighed 762 grams. It appeared normal and this abnormality was not detectable except on palpation. The central portion of the sternum which is normally straight and sword-like in structure and is cartilaginous at this age, was found to be pliable, unusually broad and divided to form two ends instead of one.

The cockerel was killed and the skeleton removed and prepared for anatomical study. All the bones were examined and found to be normal except the sternum (Figure 7, right). For comparison, a full brother weighing 810 grams and hatched the same day, was physically examined and found to be normal. This cockerel was killed and the skeleton prepared as previously described. The bones including the sternum, of this cockerel were normal (Figure 7, left).

The sternum of the abnormal cockerel

*The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

was found to be partially divided anteriorly and posterially. The keel-ridge or cresta sterna was slightly crooked. The area of calcification was unusually broad and extended approximately half the length of the sternum. The remaining cartilaginous part of the sternum continued to increase in width and divided into two horn-like parts, as contrasted with the normal sword-like process of the sternum. At the anterior end of the sternum, the quadrilateral curved plate which forms the main body of the sternum was also divided and an oval opening was found in the center.

In 1924 Dunn* described a keel-less cockerel. He also mentions "a White Leghorn hen in which the breast bone appeared to be split or indented at the keel, but the whole bone with the exception of keel was present." These descriptions differ from that of the sternum described herein, and are the only descriptions found in the available literature concerning the point in question.

The cockerel was well nourished and no evidence of rickets or faulty metabolism was observed. This incident presents another rare case of deviant development,—possibly accidental.

*DUNN, L. C., *Jour. of Heredity* 24:307. 1924.

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Statistical Cookbookery

(*Continued from page* 51)

"effect" variance to be the first tested results in roughly doubling the "significance" in subsequent tests; that is, the probability read from the table is markedly too small.

There are less important defects also, of various kinds, a particularly frequent one being the sweeping or extravagant statement, such as that the variance is the best measure of variability and that $r = 0$ means no relation at all. The minimum class expectation frequency for chi-square calculations should be set lower than 5, and the continuity correction should be restricted to cases of but one degree of freedom. Many worthless digits are retained in the worked examples. The typography and printing leave much to be desired; besides many last-place errors, there are several gross errors in the tables. Textual statements of statistical significance are not always in agreement with the actual results of the corresponding tests.

To summarize, this book will be helpful to many who desire further understanding of the form and arithmetic of the analysis of variance and of factorial design and confounding, but (as always) the reader should be alert for erroneous or unqualified statements. Other books will be needed if a full treatment of statistics of attributes is desired.

H. W. NORTON
U.S. Atomic Energy Commission

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Symposium on the Genetics of Cancer

A SYMPOSIUM on the Genetics of Cancer, organised by the Genetical Society of Great Britain and the British Empire Cancer Campaign, is being held in June of this year at the following times and places.

Thursday, June 24th, 10 a.m.—Royal Society of Medicine, 1 Wimpoole Street, W. 1.
Inheritance of Cancer in Animals.

Thursday, June 24th, 2.30 p.m.—Medical Society of London, 11 Chandos Street, W. 1.
Virus and Carcinogen Induced Mutations.

Friday, June 25th, 10 a.m.—Royal Society of Medicine, 1 Wimpole Street, W. 1.
Virus and Carcinogen Induced Mutations (continued) and
Inheritance of Cancer in Animals (continued).

Friday, June 25th, 2.30 p.m.—Royal Society of Medicine, 1 Wimpole Street, W. 1.
Inheritance of Cancer in Man.

A number of American, European and British scientists will read papers. The meetings will be open to all interested in the subject.

INHERITANCE STUDIES WITH THE TROPICAL GRAPE

JOSEPH L. FENNELL*

THIS paper is the first report on the development of a promising new class of fruits — the tropical and subtropical vineyard grapes. It is a résumé of genetic, physiological and pathological observations made during a period of twelve years on a group of wild tropical species of grapes previously little known or completely unknown to horticulture.

The tropical grapes are genetically and taxonomically distinct from temperate zone species. Many of the problems experienced in their horticultural improvement have proved to be peculiar to their climatic and specific background, though much that is learned in regard to them is applicable to grape improvement in general.

Little is known regarding genetics of the genus *Vitis,* which is difficult to study because of its polygamo-dioecious heterozygous constitution. The many small chromosomes, the wide range of variability in character expression, frequent natural hybridity, and a long time-interval between generations, present numerous problems. Even in the temperate zone, the grape breeder must contend with many special problems. In working with the tropical species the complexities are increased by numerous physiological specializations which characterize the parent species.

It has been necessary to employ in our studies species adapted to widely different climates. The tropical species have furnished the necessary adaptability, and the temperate-zone cultivated varieties have been the source of high fruit qualities. That is to say, the parents used heretofore in making crosses were either strictly temperate, or, as with oranges, avocadoes, etc., wholly tropical or subtropical in their adaptation. The extensive hybridization of tropical and temperate species, which has been the basis of the present project, seems never to have been undertaken before.

The Tropical Species

Though all of the "tropical" species listed below are not found within strictly tropical latitudes, they are indigenous to essentially tropical climates and their reactions are tropical.

The most widely distributed and commonly known tropical species is *Vitis tiliaefolia* H. B. (*V. caribaea*), the "Agras" or "Uva Silvestre" of the jungles of Central and South America. This species is indigenous to medium and low elevations from Cuba and Mexico south to the Equator. Its "basswood" - like leaves make it easy to recognize. The species is exceedingly prolific of large and compound clusters; the small acid berries are of very poor quality. Despite this, it has been a valuable parent in breeding some of our promising new horticultural varieties.

The Callosa grape (*Vitis shuttleworthii* House) is known to occur wild only in the swamps in the southern half of the Florida peninsula. The vines are vigorous, healthy and prolific. Its small dark green leaves are typically white- or tan-felted beneath. Its fruit clusters are small and compact; the large, maroon to dark red berries often measure above three-fourths of an inch in diameter. In dessert quality it varies from fair to poor. The wild vines of this species used in these experiments were collected in extreme southern Florida.

Another species, extensively em-

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ECOLOGICAL QUANDARY

Figure 8

Vine of the North American cultivated variety Golden Muscat in premature dormancy and with immature fruit. Note the short weak canes.

PROMISING HYBRID

Figure 9

Vine of one of the new hybrid tropical selections with ripe fruit and ample healthy foliage.

GROWTH-SCHEDULE UPSET

Figure 10

An illustration of prolonged dormancy affecting all but the terminal buds. "Bull-canes" are in the making and only a slight fruit crop is in prospect. This adverse reaction to a tropical environment is a heritage from the temperate climate parent.

LUXURIANT ADAPTED GROWTH

Figure 11

Normal and equal growth from many buds is shown by *Vitis shuttleworthii,* one of the wild tropical species.

ployed in this breeding project, is the rare and little known *Vitis gigas* Fen., a new species recently discovered along the Atlantic coast of southern Florida. Indications are that this may have been a comparatively recent immigrant from the Bahamas where a similar grape is reported to occur. The vines are strong and vigorous, producing large sharply lobed leaves with acute or attenuate apexes. They are thinly tan-felted beneath.

Fruit clusters of this species are usually large and well compounded, having medium small (11 to 14 mm.) good quality black berries with bloom. The species seems to prefer strand locations and dry slightly alkaline soils.

Two subtropical forms of the Aestivales series of species, *V. rufotomentosa* and *V. smalliana,* have likewise been used, though to a lesser extent than the three first mentioned.

Climatic Influence on Growth and Dormancy

Perhaps the most important physiological maladjustments encountered in hybridizing temperate climate horticultural varieties, such as Concord, Tokay or Beacon, with a tropical* species (*V. shuttleworthii* or *V. tiliaefolia*) were those connected with the normal growth and rest cycles. These apparently result from the individual or combined action of temperature, of humidity of air or soil, or perhaps of photoperiodism—especially during the dormant season.

Practically all of the European and North American grapes have a low temperature requirement for the rest period. With some this is so pronounced as to inhibit spring growth when winter temperatures do not fall far enough. With others, as the varieties Niagara, Isabella, Golden Muscat and similar hybrids with *vinifera,* as well as with certain southern types of the *vinifera* species, this phenomenon may be evidenced by abnormal and delayed spring foliation from fully dormant canes. Weak early growths of poor color, or activation of only a few dormant buds, with subsequent development of abnormally large "bull canes," may give additional evidence of insufficient chilling. The yield under these conditions is usually small and of mediocre or poor quality.

By contrast, the lowland tropical wild species have little or no chilling requirement, as would be expected in view of their environment.

In temperate climates the important function of starch hydrolysis in dormant tissues is aided by cold weather.[2] In the warmer regions, where chilling does not occur, this transformation must be accomplished in other ways which the tropical species are normally adapted to do. For this reason, more than any other, we must resort to the wild tropical species as a foundation for warm climate viticulture.

When the temperate climate varieties such as Niagara, Concord, Isabella, the Munson hybrids, or even most *vinifera* varieties, were planted in the humid tropical climate of lowland Costa Rica (latitude 10 degrees North; elevation 1,900 ft.) or even in southern Florida, the first season's growth was by no means normal. Most of the temperate North American sorts produced mature leaves and canes in an abnormally brief time after budding, lapsing into dormancy usually in the very middle of the growing season. Our observations strongly indicate that this is due, in part at least, to photoperiodic influences. In any case, two or more prunings a year have been necessary to keep the plants even partially in harmony with the seasons.

When lowland tropical species, having

*In this project the dividing-line between tropical and temperate conditions has been determined mainly by temperature. In latitudes 25° - 30° N., the southern edge of the temperate zone has been determined by the following criteria: occasional (once in five years) extreme minimum temperature not lower than 27° F.; typical annual minimum not lower than 32° F.; mean temperature during winter or dormant season not lower than 65° F.; mean minimum temperature during growth season not lower than 70° F. Where temperatures are above this and the latitude less, the climate would be considered as subtropical or tropical; if temperatures range lower, and latitude higher, the climate would be considered temperate.

LARGE CLUSTER—SMALL BERRIES

Figure 12

The large compound cluster and small berries of *Vitis tiliaefolia,* a native of tropical America. The rule is one foot long. This species has been a valuable source of adapted genes in the production of superior hybrids.

DISEASE RESISTANCE FROM OVERSEAS

Figure 13

An illustration of the inheritance of disease resistance in a hybrid. The leaves on the left are badly attacked by leaf-spot. They are from native American types. The resistant foliage at right is from hybrids of these species with *V. vinifera* from Europe.

SUPERIOR SELECTIONS

Figure 14

The large compound cluster and medium size grapes (left) were produced by a complex hybrid combining genes from several tropical species and the Malaga-Pense variety of *Vitis vinifera.* These large and tasty grapes (right) are a rare and desirable combination of genetic factors in a complex hybrid. Fruit size, quality and flavor closely resemble the European grape, while vine adaptability and foliage is mostly that of the wild tropical species.

no chilling requirement and well adjusted to short-day periods, were hybridized with temperate climate species, various complications arose. With few exceptions, the poor adaptability of the temperate climate parents to tropical environments has been at least partially dominant in first generation progeny. In most cases, it has been necessary to cross these partially adjusted F_1 hybrids back on the wild tropical species in order to attain a satisfactory degree of adaptability. It has been difficult to attain at the same time high fruit quality.

In a population of 1,300 F_2 and double cross progeny, all with tropical $\times$ temperate parentage, there was no clear-cut segregation with respect to chilling requirement or general adaptability. This might indicate that these differences are conditioned by complex, multiple-gene heredity. The percentage of individuals that showed adaptability equal to that of the tropical parent species was so small and hard to classify that no definite conclusions were suggested regarding the number of genes involved.

When these F_1 hybrids were crossed back to the tropical species, not more than ten per cent of the progeny has equalled the tropical parent in adaptability to warm dormant periods. It is understood, of course, that in such complex quantitative expressions many indirect and possibly interacting factors may be concerned. It is possible, also, that such modifying circumstances as variation in soil, atmosphere and reaction to disease may be involved. The selection of strains having good adaptability with even mediocre quality fruit has been the most difficult aspect of the entire project.

Disease Resistance

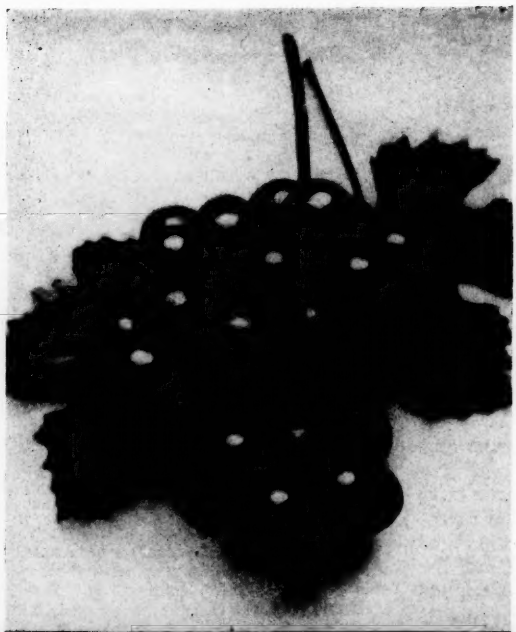
Freedom from disease is a condition usually difficult to attain. Often it is correlated with various climatic and physiological influences such as temperature, humidity and perhaps even with photoperiodism as it effects the maturity of tissue cells. Results of our experiments have indicated that premature defoliation of North American grapes in the tropics is often as much the result of early cell senility as of direct fungus attack.

Four important vine diseases of the tropics, downy mildew, rust, anthracose and *Cercospora* leaf-spot, with several others of minor consequence, have been studied for twelve years of the author's experiments in Costa Rica, Puerto Rico, and southern Florida. No correlations in resistance involving any two of these diseases could be found. A wild species or a hybrid variety may have good resistance to any one, two, or three of the

TABLE I.—Species of pronounced importance to warm climate viticulture and their pertinent characteristics. 0=completely susceptible or unsatisfactory. 10=completely resistant or satisfactory.

	Resistance to					Adaptability to		
	Rust	Anthracnose	Downy† mildew		Alternaria leaf-spot	Warm dormant season	Wet Soils	Dry Soils
Tropical Species			A	B				
V. shuttleworthii, House	9	8	6	9	9	10	10	7
V. simpsoni, Munson	9-10	7	8	9	8	10	10	5
V. tiliaefolia, H. & B.	9-10	7	8	9	9	10	10	6
V. gigas, Fennell	6	10	7	9	7	9	7	9
V. rufotomentosa, Small	6	10	8	9	7	8	6	9
V. smalliana, Bailey	6	10	8	9	7	8	6	8
**V. munsoniana*, Simpson	10	7	10	10	7	9-10	9	9
**V. popenoei*, Fennell	10	6	10	10	8	10	9	5
Temperate Species								
V. labrusca, L.	3	10	8	7	4	1	6	4
V. vinifera, L.	0-2	0-3	0-9	0-2	4-8	0-5	3	8
V. bourquina, Mun	5	8	5	5	4-5	3	4	7
V. lincecumii, Buckley	4	8	8	8	4	2	4	8
V. champini, Planch	4	7	8	9	3	3	3	9
**V. rotundifolia*, Michx	10	8	10	10	6	3	7	6

*Sub-genus muscadinia
†A—resistant in young growth; B—resistant in mature growth.



HORTICULTURAL VICTORY

Figure 15

Superior strain selected from *Vitis shuttleworthii*. Grapes average seven-eights of an inch in diameter.

EXTREME FECUNDITY

Figure 16

An F_1 hybrid of *Vitis tiliaefolia* × *V. vinifera* at Turrialba, Costa Rica. These plants are resistant to the important diseases and are very sturdy, with large crops of fruit.

QUANTITY AND QUALITY

Figure 17

A derivative of the selection shown in Figure 9 crossed with a wild hybrid from southern Florida. This plant has three-fourths tropical parentage involving at least three species. Fruit from this vine is shown in Figure 14 (right).

pathogens and complete susceptibility to all the others. Or in rare cases it may possess pronounced resistance to all of them. Studies[3] in the United States have likewise shown this in part. In our new tropical varieties resistance to each pathogen has had to be obtained separately, and usually from a different specific source. No linkages of resistance to one disease with susceptibility to another have been experienced.

Table I lists the known *Vitis* species of greatest promise to tropical grape development, together with ratings for their relative resistance to the most destructive fungi. Their adaptability to warm dormant seasons and to wet and dry soils is likewise indicated.

The ratings in Table I are made on a basis of 0 to 10 for the principal modifying influences at Turrialba, Costa Rica, and at Miami, Florida. 0 would indicate the extreme in susceptibility or poor adaptability, and any rating below 2 would be too poor for survival; 4, roughly, forms the dividing line in relation to satisfactory behavior, any rating below this figure being viewed as entirely unsatisfactory. To be considered as satisfactory for growth and production of fruit under humid tropical conditions a species should have no rating below the level of 6. Those rating six or higher in all categories have proven to be entirely satisfactory.

Notes on Disease Resistance

Rust

The fungus known as grape rust (*Physopella vitis* [Thuem.] Arth.) is one of the most destructive vine diseases of the American tropics. In our Institute vineyard where some leaves persist on the vines throughout the year, this fungus remains almost perpetually in the uredinal stage.

Varieties derived mostly from *Vitis labrusca, V. vinifera, V. aestivalis,* or nearly any of the temperate species, have been most susceptible to *Physopella.* On the other hand, three species of the tropical group, *V. tiliaefolia, V. simpsoni* and *V. shuttleworthii,* have been consistently almost immune to it. Breeding experiments conducted by the author have shown that the first two species transmit resistance as a dominant to mature progeny and that it is controlled by relatively few genes. This situation appears to parallel the mature plant resistance to *Puccinia* in wheat.[4]

When *V. tiliaefolia* was hybridized with *V. vinifera,* which appears to be homozygous for susceptibility, all resultant mature F_1 progeny have been practically immune. When these rust-free F_1 plants were again crossed with moderately susceptible species, 24 from a total population of 38 have been relatively free of the fungus in mature specimens.

From a population of 28 F_1 progeny of *V. simpsoni,* × *V. rufotomentosa* and × *V. shuttleworthii,* all mature plants have shown virtual immunity. Inheritance in the F_2 generation in this case has not been studied. When the muscadines, *V. popenoei* and *V. munsoniana* were crossed with Euvitis varieties homozygous for rust susceptibility, all F_1 progeny were immune.

V. tilifolia and *V. simpsoni* are of value to the breeder as a source of high resistance against *Physopella.*

Vitis shuttleworthii has been virtually free from rust, though it is not so strongly resistant as the two preceding species. Its resistance appears to be conditioned by multiple factors. When this moderately resistant species was crossed with very susceptible varieties of *V. labrusca, V. vinifera* and *V. aestivalis,* all F_1 progeny have been susceptible to varying and intermediate degrees.

Downy Mildew

Downy Mildew (*Plasmopara viticola,* [B. & C.] Berl. & Det.) is perhaps the most complex and at times the most misunderstood of all fungus diseases of *Vitis.* In the humid tropics it may be destructive as in the temperate regions. Resistance to this pathogen presents confusing problems. It is a common occurrence that varieties, thought to be resistant, become susceptible when moved to a different climate or location. A variety may show striking freedom from mildew, even when surrounded by badly infested plants only to be severely attacked at a later date. In such cases, the question obviously is not one of true resistance, but what would seem more likely, that of environmentally induced protection, or perhaps even of genetic protection during some limited growth stage.

It is established[7] that variations in temperature and soil moisture, when correlated with atmospheric humidity, exert a powerful influence in respect to infestations of *Plasmopara.* Such influences obscure the understanding of just what constitutes true or genetic resistance.

No known species of true bunch grape (*Euvitis*) is completely immune to downy mildew. Probably the nearest approach can be found in some types of the *Aestivales* series, as in forms of *V. rufotomentosa.* Studies by the author indicate that various widely different factors exist within the genus that may contribute toward an over-all resistance when combined in a single hybrid plant.

In Table I it will be noted that resistance

to *Plasmopara* is expressed in two distinct ways: (a) resistance in newly developed tissue; and (b) resistance in mature tissue. Each is equally important.

When mature plants of *V. shuttleworthii* grew intertwined with those of Feher Szagos and Cabernet Sauvignon (*V. vinifera*) young leaves and flower buds of the first species have been badly attacked while at the same time those of the two *vinifera* varieties in a comparable growth stage showed no sign of infection. Later in the season, when all leaves were mature, the reverse condition existed.

When these kinds, resistant and susceptible in different ways, were crossed, selections were obtained that showed better resistance, as expressed in practical results, than was possessed by either parent or parent species.

The dual-phase resistance to *Plasmopara* might be accounted for in this manner: Some forms of susceptible European grape are free of downy mildew in early growth, probably due to lack of food in the newly formed cells and to closed stomata.[5] Mature foliage of the *V. shuttleworthii*, though rich with food and obviously at times with open stomata, would seem to be resistant as a result of chemical or physical constitution of protoplasm and cell walls.

In these studies, resistance to downy mildew in stage (a) seems to be inherited mostly as a dominant, while in stage (b) it would appear that complex quantitative factors are involved anl inheritance is, at least in part, recessive.

A still different expression of resistance to this disease has been observed in the muscalinia section of the genus. When *V. shuttleworthii* was hybridized with *V. munsoniana*, the factors of resistance in both (a) and (b) stages possessed by the muscadine staminate parent have been completely dominant. Whether this dominance is a simple or multiple factor inheritance could not be determined, since these hybrids have been sterile and no F_2 progeny was obtained. In most other plant characters this complete dominance has not been manifest.

Anthracnose

Resistance to anthracose (*Elsinoe ampelina* [DeBy.] Shear.) is closely conditioned by multiple factors. The majority of our experiments indicate that no more than two or possibly three, major factors condition susceptibility. Results obtained by the author have shown susceptibility usually to be dominant, with resistance transmitted as a recessive, which segregates in the second and later generations. The results of some of these studies are given in Table II.

The majority of wild tropical species show moderate to good resistance to anthracnose, but when they were crossed with the highly susceptible *V. vinifera*, susceptibility of the latter in all cases was transmitted as a dominant to the F_1 progeny. (See Table II).

Leaf Spot

Leaf spot diseases of the *Alternaria* and *Cercospora* group are very destructive to some species when grown in tropical America. The temperate North American species, *labrusca, aestivalis,* and *lincecumii,* and most of those native to the dryer southwest regions, have been severely attacked as is the case in some of the humid sections of the southern United States.[4] When these leaf-spot-susceptible species were crossed with *V. tiliaefolia* and *V. shuttleworthii,* or with some forms of *Vitis vinifera;* hybrids with satisfactory resistance were obtained. Since most tropical species have good resistance to this disease, it has not been of major importance in our experiments.

Diseases of Minor Importance

The disease known as black-rot (*Guignardia bidwellii* [Ell.] V. & R.) has not been

TABLE II.—Inheritance of susceptibility to anthracnose in certain crosses.

Homozygous for resistance (unless indicated otherwise)	Susceptible varieties	Progeny		
		Badly suscep.	Mod. suscep.	Resistant*
1. No. 9 (*V. rufo.*)	× Alphonse Lavellee (vin.)	23	0	0
2. *V. tiliaefolia*	× Alphonse Lavellee (vin.)	14	0	0
3. No. 5 (*V. shut.*)	× Feher Szagos (vin.)	13	0	0
4. No. 9 (*V. rufo.*)	× Alexandria, Red Muscat, Sultanina, Feher Szagos, Lignan Blanc, Chasselas Golden, Black Monukka, etc. (vin.)	187	0	0
5. No. 23 (*V. shut.* × *V. rufo.*)	× No. 105 (cross no. 1)	42	76	37
6. No. 23	× No. 106 (cross no. 2)	2	5	3
7. No. 106 (suscep.)	× selfed	5	0	0
8. No. 23	× Gros Colman (vin.)	12	0	0
9. No. 6 (*V. rufo.* × *V. shut.*)	× Malaga (vin.)	4	3	0
10. No. 18 (*V. gigas*)	× Malaga (vin.)	14	5	0
11. No. 18	× (No. 9 × Lomanto) slightly suscep.	6	0	4
12. No. 23	× Portland (*V. can.* × Rommel, both resistant)	0	0	27
13. No. 18	× Extra (resistant)	0	0	7
14. No. 18	× Cabernet Sauvignon (vin.)	64	0	0

*Even resistant kinds may occasionally show a few lesions on tendrils and petioles, but if of no consequence the variety is considered resistant.

a problem thus far with the tropical species. The relation of this disease to these new sorts will need to be more fully determined, but as yet there is no indication that it will be a limiting factor.

The so-called ripe and bitter rots (*Glomcrella cingulata* [Ston.] Sp. & Von. S) and (*Melanconium fuligineum*) [Scrib. & Viala] Cav.) have not seriously attacked the best selections. Our observations suggest that these diseases prefer fruit with a high acid content and a low percentage of sugar. Selections that have produced berries rich in sugar and with the moderately firm fruit texture of *vinifera* have seldom been attacked. It is not anticipated that these rots will become a problem with our better tropical kinds.

Powdery mildew (*Uncinula necator* [Schw.] Burr.) is almost universally prevalent, though in our experience its ravages have been confined to the Old World temperate varieties and it has not been a major hazard to the tropical varieties or species.

Neither Phylloxera nor Nematodes have been a problem during our present investigations. Although both parasites are of common distribution and galls of the former may be found on the foliage of wild vines, the root systems of the tropical types have been unusually healthy. Obviously, a high degree of genetic resistance to these most destructive pests is an inherent characteristic in most of our material.

In the literature of grape breeding it has been commonly stated that characteristics of *V. vinifera*, always are dominant when crossed with western hemisphere types, and for this reason carry with them into the hybrid progeny a host of weaknesses. Our own results indicate that this is only partly true. Although *vinifera* generally has impressed its characters upon the progeny—good as well as bad—just how bad such inheritance proves to be has depended upon the nature of the combination. As an example, when we crossed a form having high susceptibility to downy mildew in either growth stage with another of the same type nearly all progeny were badly attacked. Little of value could be expected by this approach, yet it has been a common error that has prevented best results in the hybridizing of *Vitis vinifera* in North America.

General Inheritance

Most of the data available on the genetics of *Vitis* are based on such a small and specialized part of the genus as to have limited value in general application. As a genus the grape is polygamo-dioecious in flower function and thereby has a rather heterozygous genetic constitution. In the *Euvitis* (bunch grape) section the somatic chromosome number is 38. Since all species freely intercross and since dominance is variously expressed, the difficulty of predicting inheritance in new hybrids is obvious.

In the present work with tropical species, as in other comparable efforts, it has been found that certain parental combinations produce much better progeny than others. To isolate these complementary genotypes from the vast quantity of breeding material is a large undertaking in itself.

Inheritance of flower morphology and function has proved to be of a complex nature and widely varying progeny ratios are obtained according to parentage. When functionally hermaphrodite types were crossed with functionally pistillate forms, results in many cases have given considerably more pistillate than hermaphrodite progeny. In other cases, ratios as high as three hermaphrodite to one pistillate have been obtained in relatively large populations. In rare cases, all of the progeny have been hermaphrodite, which indicates homozygosity for this condition on the part of one parent as has been shown by Oberle.[6] The author has given considerable study to this subject and hopes to publish his findings in the near future.

Transmission of Fruit Character

Fruit size is one of the most important and at times most difficult qualities to obtain. In crosses of North American (*V. labrusca*) and the European species this matter has seldom been given serious consideration, since both parent types produced large fruit. As a result there are little if any data available on berry size inheritance in grapes.

When small fruited species must be used to obtain certain essential vine characters the question of berry size inheritance becomes extremely important. Our experiments have shown that berry size is controlled by several factors and that wide variation in its expression is shown in the F_2 and later generations.

When *Vitis rufotomentosa*, with a maximum berry diameter of 12 mm., was crossed with varieties of *V. vinifera* and *V. labrusca* of 20 mm. diameter, ber-

ries of the F_1 hybrids (more than 400) ranged between 13 mm. and 16 mm. maximum diameter. When we crossed several of these 13 mm. F_1 hybrids with similar though unrelated F_1 plants, berries of the resultant double cross progeny ranged between 11 mm. and 21 mm. maximum diameter. Approximately eight percent of a population of 135 fruiting plants of such crosses have produced berries larger than those of either F_1 parent. Less than two percent of the progeny have equalled either of the larger-berried grandparents in respect to fruit size. That would indicate that three or more factors condition berry size in these instances.

Of fruit flavor, considerable dominance often is shown in first generation hybrids. Ordinarily, the European grape transmits superior fruit qualities to hybrids with American species though the percentage of individuals showing this quality has varied in different crosses.

When musky-flavored *V. labrusca* varieties were crossed with the clear flavored *V. gigas* and *V. rufotomentosa,* many of the F_1 progeny possessed the full *labrusca* flavor and most of the plants had it to some extent. When the same *labrusca* varieties were crossed with the mild flavored *V. shuttleworthii,* equal dominance in flavor expression has not been shown; a high percentage of the progeny possessed little or no pronounced flavor of any kind. From this it is believed that certain flavor-inhibiting genes may be carried by *shuttleworthii.*

Berry texture is as important as flavor, and in general, the American species are inferior in this respect. For the more refined qualities of texture we have had to rely upon the disease susceptible *V. vinifera.* In these crosses it has been difficult to separate good texture from undesirable vine weaknesses. Many genes appear to influence texture of pulp and our results suggest that in most cases the tough inner-pulp characters of American varieties are at least partially dominant when crossed with *V. vinifera.* Selection of desirable combinations has been possible in the course of our present work.

With the tropical species adherence of the berry to the pedicel is usually adequate in favorable contrast to most of the temperate-zone American species.

Sugar content of berry depends upon both genetic and environmental factors. The *aestivalis* series of species (*V. gigas, V. smalliana,* and *V. vinifera*), have been the best source of this character. Though the sugar content of *V. shuttleworthii* and *V. tiliaefolia* is comparatively low, some of the complex hybrids between them and the *aestivalis* group have had remarkably sweet fruit, even under wet weather conditions. Sugary raisins have been made from a few of these selections in Costa Rica.

Shape, type, and size of cluster is apparently controlled by only a few genes. The situation is complicated by the influence of various environmental conditions. In many crosses inheritance of cluster type and size is variable, with some dominance of a tendency to form large compound bunches. However in other crosses the small compact type of bunch has been dominant.

Seedlessness is an important asset for a table grape. Seedlessness appears in some first generation progenies, to an extent of about 12 percent when one parent is seedless.[8] In our tropical selections this has not been an easy character to obtain. The few popular European varieties that produce seedless fruit (Sultanina, Black Monukka, etc.) are unusually susceptible to downy mildew, especially through the early growth stage. During these experiments we have grown hundreds of hybrids between the seedless *vinifera* and the tropical species. Nearly all have been badly attacked by disease and few have fruited. From crosses with certain seeded *vinifera* types, healthy F_1 progeny have been ob-

TABLE III.—Ideal specific background for a tropical bunch grape.

Species (select types)	Species proportionment	Fruit quality	Fruit size
V. shuttleworthii	5/16	pulpy, acid	18-20 mm.
V. tilaefolia or,			
V. simpsoni	4/16	poor, acid	8 mm.
V. gigas	3/16	sweet, good	12-14 mm.
V. vinifera	3/16	sweet, good	16-22 mm.
V. labrusca	1/16	pulpy, fair	18-20 mm.

tained which show seed abortion of 70 to 80 percent. It is believed that we may eventually obtain complete seedlessness in an otherwise satisfactory variety.

In general small seeds have been recessive to large seeds, with a complete dominance in F_1. When the large seeded variety Alphonse Lavallee (*V. vinifera*) was crossed with the very small seeded *V. tiliaefolia* all the F_1 had seeds as large, or nearly as large, as the *vinifera* parent. Some of these plants had berries only slightly larger than those of the very small fruited parent. No pronounced correlation existed between size of seed and size of berry.

The ideal tropical vineyard grape, in contrast to the situation in temperate zones, can scarcely be expected to emerge as a selection from a species or even from the progeny of a simple species cross.

So far as there is record this is the first attempt to develop tropical grape varieties through inter-breeding of wild tropical species. Success will not come through "improving" any of these wild species. Through an intricate compounding of several of these with temperate-zone varieties we are developing a "cultivated" race altogether different from those now known to viticulture.

On the basis of the author's experiments, he believes that the goal will be achieved by a combination of at least five—possibly six—species. The results obtained to date allow us to name the most promising parental species, and also to predict about the proportion in which they will be represented in the final selections. (See Table III). The illustrations accompanying this article show the extent to which the goal has now been achieved, and point to complete success in the not very distant future.

Summary

The development of new tropical grape varieties has involved many unusual and complex cultural factors: Warm dormant seasons, continuous epidemics of several unusual vine diseases, extremes of rainfall and drought, relatively short day-lengths, and often strongly acid soils, have imposed many new problems. A survey is given of important wild species and their adaptability. The genetic basis for these responses has been studied with especial reference to the more destructive grape diseases and their inheritance. Inheritance of such characters as fruit size and flavor, cluster and seed type are reported.

The development of tropical viticulture, as seen from our present viewpoint, is discussed. *Vitis shuttleworthii, V. gigas,* and *V. tiliaefolia* in combination with a slight amount of *V. vinifera* appears to offer the greatest promise as foundation material.

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